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(FILE 'HOME' ENTERED AT 10:34:55 ON 03 AUG 2000)

FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH' ENTERED AT 10:35:17 ON 03 AUG 2000

L1 6635 S NEUROECTODERMAL(5A)(TUMOR OR CANCER OR NEOPLASIA)
L2 68 S CHLOROTOXIN
L3 0 S L1 AND L2
L4 2389718 S TUMOR OR CANCER OR NEOPLASIA
L5 26 S L2 AND L4
L6 11 DUP REM L5 (15 DUPLICATES REMOVED)
L7 52241 S FLUROSCHROME OR BIOTIN OR COLORIMETRIC(W)AGENT
L8 176691 S FLUORESCENT(W)MICROSCOPY OR ELIZA OR ELISA OR FACS
L9 226144 S L7 OR L8
L10 0 S L9 AND L6
L11 0 S L9 AND L2

=> d bib ab 1-11 16

L6 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2000 ACS
AN 1999:330028 CAPLUS
DN 130:335024
TI Method of diagnosing and treating gliomas
IN Ullrich, Nicole; Sontheimer, Harald W.
PA UAB Research Foundation, USA
SO U.S., 34 pp.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 2

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|---|
| PI | US 5905027 | A | 19990518 | US 1996-774154 | 19961226 |
| | US 6028174 | A | 20000222 | US 1997-980388 | 19971128 |
| PRAI | US 1995-9283 | | 19951227 | | |
| | US 1996-774154 | | 19961226 | | |

AB The present invention provides a recombinant toxin and monoclonal antibody which specifically binds to glial-derived or meningioma-derived **tumor** cells. Also provided are various methods of screening for malignant gliomas and meningiomas. Further provided are methods of treating malignant gliomas, including glioblastoma multiforme and astrocytomas.
RE.CNT 2
RE
(1) Ullrich; Am J Physiol 1996, V270(5, pt 1), PC1511
(2) Ullrich; Neuro Report 1996, V7(5), P1020 MEDLINE

L6 ANSWER 2 OF 11 MEDLINE DUPLICATE 1
AN 1999337948 MEDLINE
DN 99337948
TI Modulation of glioma cell migration and invasion using Cl(-) and K(+) ion channel blockers.
AU Soroceanu L; Manning T J Jr; Sontheimer H
CS Department of Neurobiology, The University of Alabama at Birmingham,

Birmingham, Alabama 35294-0021, USA.
NC NS36692 (NINDS)
SO JOURNAL OF NEURO[redacted]ENCE, (1999 Jul 15) 19 (14) 59[redacted]54.
Journal code: JDF. ISSN: 0270-6474.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199910
EW 19991001
AB Human malignant gliomas are highly invasive **tumors**. Mechanisms that allow glioma cells to disseminate, migrating through the narrow extracellular brain spaces are poorly understood. We recently demonstrated expression of large voltage-dependent chloride (Cl(-)) currents, selectively expressed by human glioma cells in vitro and in situ (Ullrich et al., 1998). Currents are sensitive to several Cl(-) channel blockers, including **chlorotoxin** (Ctx), (Ullrich and Sontheimer; 1996; Ullrich et al; 1996), tetraethylammonium chloride (TEA), and tamoxifen (Ransom and Sontheimer, 1998). Using Transwell migration assays, we show that blockade of glioma Cl(-) channels specifically inhibits **tumor** cell migration in a dose-dependent manner. Ctx (5 microM), tamoxifen (10 microM), and TEA (1 mM) also prevented invasion of human glioma cells into fetal rat brain aggregates, used as an in vitro model to assess **tumor** invasiveness. Anion replacement studies suggest that permeation of chloride ions through glioma chloride channel is obligatory for cell migration. Osmotically induced cell swelling and subsequent regulatory volume decrease (RVD) in cultured glioma cells were reversibly prevented by 1 mM TEA, 10 microM tamoxifen, and irreversibly blocked by 5 microM Ctx added to the hypotonic media. Cl(-) fluxes associated with adaptive shape changes elicited by cell swelling and RVD in glioma cells were inhibited by 5 microM Ctx, 10 microM tamoxifen, and 1 mM TEA, as determined using the Cl(-)-sensitive fluorescent dye 6-methoxy-N-ethylquinolinium iodide. Collectively, these data suggest that chloride channels in glioma cells may enable **tumor** invasiveness, presumably by facilitating cell shape and cell volume changes that are more conducive to migration and invasion.

L6 ANSWER 3 OF 11 MEDLINE DUPLICATE 2
AN 1999025865 MEDLINE
DN 99025865
TI Use of **chlorotoxin** for targeting of primary brain **tumors**.
AU Soroceanu L; Gillespie Y; Khazaeli M B; Sontheimer H
CS Department of Neurobiology, University of Alabama at Birmingham, 35294, USA.
NC RO1 NS 36692 (NINDS)
SO CANCER RESEARCH, (1998 Nov 1) 58 (21) 4871-9.
Journal code: CNF. ISSN: 0008-5472.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199901
EW 19990104
AB Gliomas are primary brain **tumors** that arise from differentiated glial cells through a poorly understood malignant transformation. Although glioma cells retain some genetic and antigenic features common to glial cells, they show a remarkable degree of antigenic heterogeneity and variable mutations in their genome. Glioma cells have recently been shown to express a glioma-specific chloride ion channel (GCC) that is sensitive to **chlorotoxin** (CTX), a small peptide purified from Leiurus quinquestriatus scorpion venom [N. Ullrich et al, Neuroreport, 7:

1020-1024, 1996; and N. Ullrich and H. Sontheimer, Am. J. Physiol. (Cell Physiol.), 270: C1511-C1521, 1996]. Using native and recombinant 125I-labeled CTX we show that toxin binding to glioma cells is specific and involves high affinity [dissociation constant (Kd)=4.2 nM] and low affinity (Kd=660 nml) binding sites. In radioreceptor assays, 125I-labeled CTX binds to a protein with Mr=72,000, presumably GCC or a receptor that modulates GCC activity. In vivo targeting and biodistribution experiments were obtained using 125I- and (131)I-labeled CTX injected into severe combined immunodeficient mice bearing xenografted gliomas. CTX selectively accumulated in the brain of **tumor**-bearing mice with calculated brain: muscle ratios of 36.4% of injected dose/g (ID/g), as compared to 12.4% ID/g in control animals. In the **tumor**-bearing severe combined immunodeficient mice, the vast majority of the brain-associated radioactivity was localized within the **tumor** (**tumor** :muscle ratio, 39.13% ID/g; contralateral brain:muscle ratio, 6.68%ID/g). Moreover, (131)I-labeled CTX distribution, visualized through in vivo imaging by gamma ray camera scans, demonstrates specific and persistent intratumoral localization of the radioactive ligand. Immunohistochemical studies using biotinylated and fluorescently tagged CTX show highly selective staining of glioma cells in vitro, in situ, and in sections of patient biopsies. Comparison tissues including normal human brain, kidney, and colon were consistently negative for CTX immunostaining. These data suggest that CTX and CTX-conjugated molecules may serve as glioma-specific markers with diagnostic and therapeutic potential.

L6 ANSWER 4 OF 11 MEDLINE DUPLICATE 3
AN 1998161360 MEDLINE
DN 98161360
TI Expression of voltage-activated chloride currents in acute slices of human gliomas.
AU Ullrich N; Bordey A; Gillespie G Y; Sontheimer H
CS Department of Neurobiology, University of Alabama at Birmingham, 35294, USA.
NC RO1-NS31234 (NINDS)
RO1-NS36692 (NINDS)
SO NEUROSCIENCE, (1998 Apr) 83 (4) 1161-73.
Journal code: NZR. ISSN: 0306-4522.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199806
EW 19980603
AB Using whole-cell patch-clamp recordings, we identified a novel voltage-activated chloride current that was selectively expressed in glioma cells from 23 patient biopsies. Chloride currents were identified in 64% of glioma cells studied in acute slices of nine patient biopsies. These derived from gliomas of various pathological grades. In addition, 98% of cells acutely isolated or in short-term culture from 23 patients diagnosed with gliomas showed chloride current expression. These currents, which we termed glioma chloride currents activated at potentials >45 mV, showed pronounced outward rectification, and were sensitive to bath application of the presumed Cl- channel specific peptide **chlorotoxin** (approximately 600 nM) derived from Leiurus scorpion venom. Interestingly, low grade tumours (e.g., pilocytic astrocytomas), containing more differentiated, astrocyte-like cells showed expression of glioma chloride currents in concert with voltage-activated sodium and potassium currents also seen in normal astrocytes. By contrast, high grade

tumours (e.g., glioblastoma multiforme) expressed almost exclusively chloride current[redacted] suggesting a gradual loss of Na+ currents and gain of Cl- currents wit[redacted]increasing pathological tumour [redacted]ade. To expand on the observation that these chloride currents are glioma-specific, we introduced experimental tumours in scid mice by intracranial injection of D54MG glioma cells and subsequently recorded from tumour cells and adjacent normal glial cells in acute slices. We consistently observed expression of **chlorotoxin**-sensitive chloride channels in implanted glioma cells, but without evidence for expression of chloride channels in surrounding "normal" host glial cells, suggesting that these chloride channels are probably a glioma-specific feature. Finding of this novel glioma specific Cl- channel in gliomas in situ and it's selective binding of **chlorotoxin** may provide a way to identify or target glioma cells in the future.

L6 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2000 ACS
AN 1997:505749 CAPLUS
DN 127:119322
TI Method of diagnosing and treating gliomas
IN Sontheimer, Harald W.; Ullrich, Nicole
PA UAB Research Foundation, USA
SO PCT Int. Appl., 81 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI WO 9724619 | A1 | 19970710 | WO 1996-US20403 | 19961227 |
| W: AU, CA, JP | | | | |
| RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| CA 2249351 | AA | 19970710 | CA 1996-2249351 | 19961227 |
| AU 9722399 | A1 | 19970728 | AU 1997-22399 | 19961227 |
| EP 953153 | A1 | 19991103 | EP 1996-946129 | 19961227 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |

PRAI US 1995-9283 19951227
WO 1996-US20403 19961227

AB The present invention relates generally to the fields of cell physiol., neurol. and neuro-oncol. More specifically, the present invention relates to a novel method of detection of the membrane protein "glioma chloride channel" for use as a specific **tumor** marker for the diagnosis and treatment of gliomas and meningiomas. The invention describes the expression of this chloride conductance with unique properties that selectively characterizes **tumor**-derived cells of glial origin. Whole-cell patch-clamp techniques were used to characterize the biophys. and pharmacol. properties of chloride channels in primary cultures and acutely isolated cells from biopsies of human astrocytomas and established cell lines.

L6 ANSWER 6 OF 11 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1997:535069 BIOSIS
DN PREV199799834272
TI Targeting of glioma cells using **chlorotoxin**, a scorpion venom peptide.
AU Soroceanu, L. (1); Gillespie, G. Y.; Khazaeli, M. B.; Sontheimer, H.
CS (1) Dep. Neurobiol., Univ. Ala. at Birmingham, Birmingham, AL USA
SO Society for Neuroscience Abstracts, (1997) Vol. 23, No. 1-2, pp. 2448. Meeting Info.: 27th Annual Meeting of the Society for Neuroscience New Orleans, Louisiana, USA October 25-30, 1997
ISSN: 0190-5295.
DT Conference

LA English

L6 ANSWER 7 OF 11 SCISEARCH COPYRIGHT 2000 ISI (R)
AN 97:781387 SCISEARCH
GA The Genuine Article (R) Number: YB153
TI Cell cycle-dependent expression of a glioma-specific chloride current: proposed link to cytoskeletal changes
AU Ullrich N; Sontheimer H (Reprint)
CS UNIV ALABAMA, DEPT NEUROBIOL, 1719 6TH AVE S, CIRC RM 545, BIRMINGHAM, AL 35294 (Reprint); UNIV ALABAMA, DEPT NEUROBIOL, BIRMINGHAM, AL 35294
CYA USA
SO AMERICAN JOURNAL OF PHYSIOLOGY-CELL PHYSIOLOGY, (OCT 1997) Vol. 42, No. 4,
pp. C1290-C1297.
Publisher: AMER PHYSIOLOGICAL SOC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0363-6143.
DT Article; Journal
FS LIFE
LA English
REC Reference Count: 38
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB We recently demonstrated expression of a novel, glioma-specific Cl- current in glial-derived **tumor** cells (gliomas), including stable cell lines such as STTG1, derived from a human anaplastic astrocytoma. We used STTG1 cells to study whether glioma Cl- channel (GCC) activity is regulated during cell cycle progression. Cells were arrested in defined stages of cell cycle (G(0), G(1), G(1)/S, S, and M phases) using serum starvation, mevastatin, hydroxyurea, demecolcine, and cytosine beta-D-arabinofuranoside. Cell cycle arrest was confirmed by measuring [H-3]thymidine incorporation and by DNA flow cytometry. Using whole cell patch-clamp recordings, we demonstrate differential changes in GCC activity after cell proliferation and cell cycle progression was selectively altered; specifically, channel expression was low in serum-starved, G(0)-arrested cells, increased significantly in early G(1), decreased during S phase, and increased after arrest in M phase. Although the Link between the cell cycle and GCC activity is not yet clear, we speculate that GCCs are Linked to the cytoskeleton and that cytoskeletal rearrangements associated with cell division lead to the observed changes in channel activity. Consistent with this hypothesis, we demonstrate the activation of GCC by disruption of F-actin using cytochalasin D or osmotic cell swelling.

L6 ANSWER 8 OF 11 MEDLINE DUPLICATE 4
AN 96226464 MEDLINE
DN 96226464
TI Biophysical and pharmacological characterization of chloride currents in human astrocytoma cells.
AU Ullrich N; Sontheimer H
CS Neurobiology Research Center, University of Alabama at Birmingham 35294, USA.
NC RO1-NS-31234 (NINDS)
P50-HD-32901 (NICHD)
SO AMERICAN JOURNAL OF PHYSIOLOGY, (1996 May) 270 (5 Pt 1) C1511-21.
Journal code: 3U8. ISSN: 0002-9513.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199702
EW 19970204
AB Expression of voltage-activated ion channels was studied in primary cultures from seven freshly resected human primary brain **tumors**

and in an established human astrocytoma cell line, STTG1. Astrocytoma cells consistently expressed voltage-dependent outwardly rectifying currents. Currents activated at potentials > 45 mV and showed outward transients on termination of voltage steps. Currents reversed at the Cl equilibrium potential, suggesting that they were largely carried by Cl-. Altering extracellular K- or Na+ concentration did not alter currents; neither did replacement of intracellular K+ by Cs+ or intracellular Na+ by N-methyl-D-glucosamine. Anion-substitution experiments suggest the following permeability sequence, determined from shifts in tail current reversal potential: I- > NO3- > Br- > Cl- > acetate > isethionate > F- > glutamate. Currents were sensitive to the Cl- channel blockers **chlorotoxin**, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), and 4,4'-dinitrostilbene-2,2' disulfonic acid (DNDS), with **chlorotoxin** being most effective, yielding > 80% block at 590 nM. DIDS (100 microM) and DNDS (100 microM) reduced currents by 33.5 and 38.2%, respectively. Currents were also sensitive to Zn2+ (100 microM, 47% block) and Cd2- (25 microM, 42% block). Reducing extracellular Ca2+ concentration decreased outward currents by 58% and almost completely eliminated transients, suggesting that Cl- currents are Ca2+ dependent. Cl channel block resulted in altered cell proliferation as determined by [3H]thymidine incorporation, suggesting that these channels may be involved in astrocytoma growth control.

L6 ANSWER 9 OF 11 SCISEARCH COPYRIGHT 2000 ISI (R)
AN 96:372248 SCISEARCH
GA The Genuine Article (R) Number: UJ814
TI BIOPHYSICAL AND PHARMACOLOGICAL CHARACTERIZATION OF CHLORIDE CURRENTS IN HUMAN ASTROCYTOMA-CELLS
AU ULLRICH N; SONTHEIMER H (Reprint)
CS UNIV ALABAMA, NEUROBIOL RES CTR, 1719 6TH AVE S, CIRC RM 545, BIRMINGHAM, AL, 35294 (Reprint); UNIV ALABAMA, NEUROBIOL RES CTR, BIRMINGHAM, AL, 35294
CYA USA
SO AMERICAN JOURNAL OF PHYSIOLOGY-CELL PHYSIOLOGY, (MAY 1996) Vol. 39, No. 5, pp. C1511-C1521.
ISSN: 0363-6143.
DT Article; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 41
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB Expression of voltage-activated ion channels was studied in primary cultures from seven freshly resected human primary brain **tumors** and in an established human astrocytoma cell line, STTG1. Astrocytoma cells consistently expressed voltage-dependent outwardly rectifying currents. Currents activated at potentials >45 mV and showed outward transients on termination of voltage steps. Currents reversed at the Cl- equilibrium potential, suggesting that they were largely carried by Cl-. Altering extracellular K+ or Na+ concentration did not alter currents; neither did replacement of intracellular K+ by Cs+ or intracellular Na+ by N-methyl-D-glucosamine. Anion-substitution experiments suggest the following permeability sequence, determined from shifts in tail current reversal potential: I- > NO3- > Br- > Cl- > acetate > isethionate > F- > glutamate. Currents were sensitive to the Cl- channel blockers **chlorotoxin**, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), and 4,4'-dinitrostilbene-2,2'disulfonic acid (DNDS), with **chlorotoxin** being most effective, yielding >80% block at 590 nM. DIDS (100 mu M) and DNDS (100 mu M) reduced currents by 33.5 and 38.2%, respectively. Currents were also sensitive to Zn2+ (100 mu M, 47% block) and Cd2+ (25 mu M, 42% block). Reducing extracellular Ca2+ concentration

decreased outward currents by 58% and almost completely eliminated transients, suggesting that Cl- currents are Ca2+ dependent. Cl- channel block resulted in altered cell proliferation as determined by [H-3]thymidine incorporation, suggesting that these channels may be involved in astrocytoma growth control.

L6 ANSWER 10 OF 11 MEDLINE DUPLICATE 5
AN 96396940 MEDLINE
DN 96396940
TI Human astrocytoma cells express a unique chloride current.
AU Ullrich N; Gillespie G Y; Sontheimer H
CS Neurobiology Research Center, University of Alabama at Birmingham 35294, USA.
NC RO-1 NS31234 (NINDS)
P50 HD32901 (NICHD)
P20 NS31096 (NINDS)
SO NEUROREPORT, (1996 Apr 10) 7 (5) 1020-4.
Journal code: A6M. ISSN: 0959-4965.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199702
EW 19970204
AB Human astrocytoma cells were studied using whole-cell patch-clamp recording. Voltage-dependent outwardly-rectifying anion currents were identified in primary cultures of six freshly resected human brain **tumors** and in seven established anaplastic astrocytoma/glioblastoma cell lines (U251MG, CH235MG, U373MG, U105MG, D54MG, SK-MG-1, and STTG1). Anion currents were not observed in normal, non-neoplastic glial cells, nor in human **tumor**-derived cells of non-glial origin (melanoma, breast **cancer**, neuroblastoma, rhabdomyosarcoma). Currents activated at potentials > 50 mV and showed large transients upon termination of voltage steps. Currents reversed at the predicted equilibrium potential for chloride ions and could also be recorded when Cl- was replaced by F-, Br- or I-. Currents were inhibited by the Cl- channel blockers **chlorotoxin**, DIDS, and DNDS. These Cl- currents may play a role in the growth control of astrocytoma cells.

L6 ANSWER 11 OF 11 MEDLINE DUPLICATE 6
AN 96352227 MEDLINE
DN 96352227
TI Human astrocytoma cells express a unique chloride current.
AU Ullrich N; Gillespie G Y; Sontheimer H
CS Interdepartmental Neuroscience Program, Yale University School of Medicine, New Haven, CT 06510, USA.
NC RO-1 NS31234 (NINDS)
P50 HD32901 (NICHD)
P20 NS31096 (NINDS)
SO NEUROREPORT, (1995 Dec 29) 7 (1) 343-7.
Journal code: A6M. ISSN: 0959-4965.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199612
AB Human astrocytoma cells were studied using whole-cell patch-clamp recording. Voltage-dependent outwardly-rectifying anion currents were identified in primary cultures of six freshly resected human brain **tumors** and in seven established anaplastic astrocytoma/glioblastoma cell lines (U251MG, CH235MG, U373MG, U105MG, D54MG, SK-MG-1, and STTG1). Anion currents were not observed in normal, non-neoplastic glial cells, nor in human **tumor**-derived cells of non-glial origin (melanoma, breast **cancer**, neuroblastoma, rhabdomyosarcoma). Currents activated at potentials > 50 mV and showed

large transients upon termination of voltage steps. Currents reversed at the predicted equilibrium potential for chloride ions and could also be recorded when Cl- was replaced by F-, Br- or I-. Currents were inhibited by the Cl- channel blockers **chlorotoxin**, DIDS, and DNDS. These Cl- currents may play a role in the growth control of astrocytoma cells.

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